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<p>(54) Title: ASEXUAL BLOOD STAGE ANTIGENS OF <i>PLASMODIUM FALCIPARUM</i></p> <p>(57) Abstract</p> <p>DNA molecules comprising artificially constructed polynucleotide sequences substantially corresponding to all or a portion of the base sequence coding for an antigen of <i>Plasmodium falciparum</i> selected from the group consisting of the Acidic Basic Repeat Antigen Rhoptry (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of <i>P. falciparum</i> cross-reactive therewith. Such DNA molecules are capable of being expressed as polypeptide(s). Synthetic peptides or polypeptides displaying the antigenicity of all or a portion of the above antigens of <i>P. falciparum</i>. Compositions for stimulating immune responses against <i>P. falciparum</i> antigens in a mammal, comprising at least one polypeptide displaying the antigenicity of the above antigens of <i>P. falciparum</i>, together with a pharmaceutically acceptable carrier therefor.</p>		

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"ASEXUAL BLOOD STAGE ANTIGENS OF PLASMODIUM FALCIPARUM"

This invention relates to synthetic peptides and polypeptides which have antigenicity suitable for providing protective immunity against Plasmodium falciparum infections, and to methods for the production
5 thereof.

The human malaria parasite Plasmodium falciparum encodes many polypeptides that elicit an immune response in man. Recently, molecular cloning techniques have
10 facilitated the analysis of individual polypeptide antigens that are present in this complex mixture (1). Many cDNA clones encoding these antigens have been isolated by screening Escherichia coli colonies that express the cloned sequences with human antibodies. The
15 production and screening of these clones is described in detail in International Patent Specification No. PCT/AU84/00016.

The present invention is based upon the
20 identification and characterisation of further asexual blood-stage antigens of P.falciparum.

According to the present invention, there is provided a DNA molecule comprising a nucleotide sequence
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substantially corresponding to all or a portion of a base sequence coding for one of the antigens of P.falciparum described in detail hereinafter. In particular, there is provided a DNA molecule comprising
5 a nucleotide sequence characterized by at least a portion thereof comprising all or a portion of a base sequence shown in the accompanying Figures. Such a nucleotide sequence codes for a polypeptide comprising at least a portion which corresponds to a portion of the
10 amino acid sequence of an antigen of P.falciparum as described herein.

The present invention also extends to synthetic peptides or polypeptides displaying the antigenicity of
15 all or a portion of an antigen of P.falciparum as described herein, as well as to compositions for stimulating immune responses against such an antigen in a mammal, which compositions comprise at least one synthetic polypeptide displaying the antigenicity of all
20 or a portion of the antigen, together with a pharmaceutically acceptable carrier therefor. The synthetic peptides or polypeptides according to this aspect of the invention may be prepared by expression in a host cell containing a recombinant DNA molecule which
25 comprises a nucleotide sequence as broadly described above operatively linked to an expression control sequence, or a recombinant DNA cloning vehicle or vector containing such a recombinant DNA molecule. The synthetic peptide or polypeptide so expressed may be a
30 fusion polypeptide comprising in addition to a portion displaying the antigenicity of all or a portion of the antigen, an additional polypeptide coded for by the DNA of the recombinant DNA molecule. Alternatively, the synthetic peptides or polypeptides may be produced by

chemical means, such as by the well-known Merrifield solid-phase synthesis procedure.

(I) A Rhoptry Protein of P.falciparum

5 Intraerythrocytic asexual parasites of Plasmodium falciparum are responsible for the morbidity and mortality of this serious protozoal infection of man. Propagation of the asexual parasite occurs when mature schizonts rupture and release merozoites which invade
10 fresh erythrocytes. Invasion begins when the merozoite abuts an erythrocyte and re-orientates so that the apex of the merozoite is in contact with the erythrocyte membrane. Paired apical organelles called rhoptries discharge their contents prior to perturbation of the
15 erythrocyte membrane and subsequent entry of the merozoite. Rhoptry proteins have been implicated as potential protective immunogens in several systems (2, 3). A cDNA clone encoding a portion of a Mr 105,000 rhoptry protein of P.falciparum has now been identified
20 and characterised. A rhoptry protein of this molecular weight is present in several isolates of P.falciparum from widely separated geographical areas.

Several previously isolated cDNA clones expressing
25 P.falciparum antigens contained regions of tandemly repeated peptides. It has previously been shown that these repeat regions are frequently highly antigenic and are the immunodominant regions of the molecule recognized during natural infection. The clone Ag44
30 expressing part of the Mr 107,000 rhoptry protein is an example where a naturally antigenic determinant is encoded by non-repeat sequence. It is not known whether other portions of this molecule contain repeat regions. The identification of this clone allows the preparation

of monospecific reagents against this rhoptry protein which will enable tests of its function and potential as a protective immunogen.

5 Further details of the isolation and characterization of this protein will be apparent from the following detailed description, and from the accompanying Figures. In the Figures:

Figure 1 shows indirect immunofluorescence of the
 10 FC27 isolate of P.falciparum asexual blood stages reacted with human antibodies against Ag44. Fluorescein staining of A, a late trophozoite (T) and early schizont (S) exhibiting weak fluorescence excluded over the nuclei; B, a mature schizont with the predominant
 15 pattern of punctate fluorescence (left) and a multiply-infected erythrocyte exhibiting both punctate and lattice patterns of fluorescence (right); C, punctate fluorescence associated with extracellular merozoites. Paired spots of fluorescent staining occur
 20 within a single merozoite (arrowed). Inset is of a mature schizont from an adjacent field of view.

Figure 2 shows immunoelectron microscopy, using the protein A-gold technique, of a schizont of the FC27
 25 isolate of P.falciparum reacted with human antibodies against Ag44. The pear-shaped rhoptries containing antigen reacting with antibodies are arrowed.
 (Magnified x 79,000.)

30 Figure 3 shows immunoblots using human antibodies affinity-purified on the fusion protein of clone Ag44.
A. Identification of the corresponding parasite antigen in different life-cycle stages of FC27: uninfected

cells (1), rings (2), trophozoites (3), schizonts (4), merozoites (5).

B. Identification of the corresponding parasite antigen in 4 different isolates of P.falciparum grown in asynchronous culture: NF7 (1), K1 (2), FC27 (3) and V1(4).

Figure 4 is the nucleotide and amino acid sequence of Ag44. At the 5' end of the insert the first 2 bases derived from P.falciparum put the sequence in frame with β -galactosidase but are not translated here because the corresponding hybrid codon would not be found in the P.falciparum sequence.

Figure 5 shows the hybridization of Ag44 cDNA to restriction fragments of P.falciparum DNA. DNA from isolates FC27 (1), K1 (2) and NF7 (3) was cleaved with EcoRI (A) and Hind 3 (B), fractionated by electrophoresis on a 1% agarose gel, blotted to nitrocellulose, hybridized with 32 P-Ag44 cDNA and autoradiographed.

Materials and Methods

(See later)

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RESULTS

Identification of a cDNA Encoding a Rhoptry Protein

cDNA derived from the Papua New Guinea isolate FCQ27/PNG (FC27) isolate of P.falciparum was prepared as described and inserted into the expression vector λ gt11-Amp3 (1). A large number of clones expressing P.falciparum sequences were screened with human antibodies affinity purified against the FC27 isolate and seventy-eight antigen positive clones were

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identified (4). One such clone, Ag44, was shown to encode part of a rhoptry protein as follows. E.coli lysogenic for λ Ag44 were grown in liquid culture, heat-induced, lysed and coupled to CNBr-activated Sepharose. Human antibodies specific for the Ag44 fused polypeptide were affinity purified on this absorbent, and used to identify the P.falciparum protein corresponding to Ag44 by immunofluorescence and immunoblot assays.

Asynchronous cultures of the FC27 isolate were fixed to glass slides and examined by direct immunofluorescence. Proteins reactive with anti-Ag44 antibodies were localised to mature schizonts, in paired organelles within merozoites, a pattern characteristic of rhoptry proteins (Fig.1a). Little reactivity was seen with ring forms. Several different isolate of P.falciparum: - K1 from Thailand, NF7 from Ghana and V1 from Vietnam, all showed identical patterns of fluorescence.

Immunoelectron microscopy confirmed the rhoptry location of the antigen recognised by anti-Ag44 antibodies. There was heavy labelling of the pear-shaped organelles when sections of schizonts were incubated first with affinity purified human anti-Ag44 antibodies and then protein A-gold (Fig.2).

Immunoblot analysis of lysates of synchronized P.falciparum infected cells showed that the anti-Ag44 antibodies recognised 3 closely-spaced bands of Mr 107,000, 105,000 and 103,000 (Fig.3a). The higher molecular weight forms were more prominent in immature forms, and this may suggest a precursor product

relationship. A similar set of bands was recognised when lysates of several different P.falciparum isolates were probed with anti-Ag44 antibodies (Fig.3b).

5 Nucleotide Sequence of Ag44

DNA was purified from phage expressing Ag44. Only 1 insert was present and this was subcloned into the pUC and M13 vectors. The nucleotide sequence of the 494 bp R1 fragment was determined by the dideoxy method (Fig.4). There was a long open reading frame present which extended up to nucleotide 404 and was in frame with β -galactosidase, accounting for the large fused polypeptide synthesized by λ Ag44 (4). The predicted amino acid sequence is displayed (Fig.4). There are no tandemly repeated peptide elements as are commonly found in other P.falciparum antigens. The termination codon at nucleotides 405-407 presumably represents the 3' end of the coding region. This is consistent with the presence of deoxyadenosine bases present at the extreme 3' end of the DNA sequence which correspond to the poly(A) tail of the mRNA. This sequence predicted here would encode approximately 16% of the entire molecule.

Genomic Context of Ag44

DNA from three P.falciparum isolates FC27, K1 and NF7 was cleaved with EcoRI or AhaIII; size fractionated and blotted to nitrocellulose. The purified 570 bp R1 fragment of Ag44 was nick-translated and hybridized to the nitrocellulose filter. All isolates showed a common band of 1800 bp in EcoRI digests and 5000 bp in Hind 3 digests (Fig.5).

(II) An Acidic Basic Repeat Antigen (ABRA) of
P.falciparum

5 A Mr 102,000 antigen of P.falciparum, predominantly
 of schizonts, has been identified and characterized.
 Sequencing studies on 4 cDNA clones encoding parts of
 this antigen revealed blocks of hydrophilic dipeptide
 and tripeptide repeats and so the antigen has been
 designated the Acidic Basic Repeat Antigen (ABRA).

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Further details of the isolation and
 characterisation of this antigen will be apparent from
 the detailed description hereunder, and from the
 accompanying Figures. In the Figures:

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Figure 6 shows indirect immunofluorescence of
P.falciparum asexual blood stages reacted with human
 antibodies to Ag196. Single fields of view for isolate
 V1 (panels A, B) and FC27 (panels C, D) examined by
 20 fluorescein (A, C) and propidium (B, D) fluorescence the
 erythrocytes shown contain trophozoites (T) and
 schizonts (S).

Figure 7, shows immunoblots using human antibodies
 25 affinity-purified on the fusion protein of clone Ag196.

A. Identification of ABRA in 4 different isolates of
P.falciparum grown in asynchronous culture: uninfected
 red cells (1), NF7 (2), K1 (3), FC27 (4) and V1 (5).

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B. Detection of ABRA in different life-cycle stages of
 FC27: uninfected red cells (1), rings (2), trophozoites
 (3), schizonts (4) and merozoites (5).

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C. Triton X-100 - extracts of the same life-cycle stages as in (B) (lane 1 to 5). Triton-insoluble pellets were resolubilized in NaDodSO₄: uninfected cells (6), rings (7), trophozoites (8), schizonts (9) and merozoites (10). Molecular weight markers are myosin (200kD), β -galactosidase (116 kD), phosphorylase D (92 kD), bovine serum albumin (66 kD) and ovalbumin (45 kD).

Figure 8 is the nucleotide and amino acid sequence of Ag189. The start of Ag144, Ag196 and Ag 126 in relation to Ag189 are indicated by arrows and the adjacent clone number.

Figure 9 shows hybridization of Ag126 cDNA to restriction fragments of P.falciparum DNA. DNA from the 3 isolates of P.falciparum indicated was cleaved with EcoRI (1) and Aha III (2), fractionated by electrophoresis on a 1% agarose gel, blotted to nitrocellulose, hybridized with ³²P-Ag126 cDNA and autoradiographed. The P.falciparum isolates were: FC27 from Papua New Guinea; NF7 from Ghana and K1 from Thailand.

Materials and Methods

(See later)

RESULTS

ABRA is located in the mature schizont

Indirect immunofluorescence was performed on acetone-fixed, asexual blood-stage parasites using human antibodies affinity-purified on an immunoadsorbent of Ag196. The antibodies reacted strongly with erythrocytes containing schizonts and gave predominantly a lattice pattern of fluorescence which is particularly

well resolved in isolate V1 (Fig.6A). Counterstained nuclei of the developing merozoites appeared within regions that excluded fluorescein staining (Fig.6B). Little or no reactivity was seen with ring and trophozoite stages of V1.

More intense fluorescence was observed at a given antibody dilution with isolate FC27. Staining again occurred predominantly with erythrocytes containing schizonts, but there was diffuse staining in trophozoites to a greater extent than with V1 (Fig.6C). Fluorescence of the surface of infected erythrocytes was not seen when the assay was performed using unfixed cells or lightly glutaraldehyde-fixed and air-dried monolayers (5). Similar results with FC27 were obtained using mouse antisera against clones Ag196, Ag189, Ag126 and Ag203 belonging to the same serological family.

In immunoblots of asynchronous parasite preparations, affinity-purified human antibodies against clone Ag196 detected a dominant band of Mr 102,000, which did not vary between the 3 isolates NF7, FC27 and V1 (Fig.7A). The corresponding protein is approximately Mr 2,000 smaller in isolate K1 from Thailand (Fig.7A).

In immunoblots of life-cycle stages (Fig.7B) the dominant Mr 102,000 band was present in schizonts was poorly represented or absent from other stages. A weak band of Mr 230,000 was also present in schizont preparations (Fig.7B). The target antigen was recovered in Triton extracts of infected erythrocytes and no additional material was detected by anti-Ag196 antibodies when pellets were resolubilized in NaDodSO₄ sample buffer (Fig.7C).

Nucleotide and amino acid sequence

The cDNA inserts of 4 members of the Agl96-family were isolated. The insert of Agl89 was subcloned into the vector M13mp8 and its nucleotide sequence determined by the dideoxy procedure. Agl89 contains an insert of 965 bp, which has a single open reading frame extending through the whole cDNA. This frame is shown in Figure 8. All the other frames are interrupted by multiple stop codons. Agl89 is not in frame with β -galactosidase and does not produce a large fused polypeptide. A number of other clones from similar expression libraries were out of phase with β -galactosidase (6).

Hydrophilic dipeptide and tripeptide repeats predicted from the sequence of ABRA

The sequence of Agl89 from position 1 to 834 encodes predominantly hydrophilic amino acids. At the 3' end starting at position 835 extends a highly charged region which consists of 10 dipeptide repeats (Glu-Lys) and 6 interspersed tripeptide repeats (Glu-Glu-Lys). The repeat-block is flanked on either side by three glutamic acids.

Three blocks of 12 nucleotides starting from position 678 to 714 exhibits a high degree of homology. These "cryptic" dodeca-nucleotide repeats only show a minor degree of similarity on the amino acid level. Asparagine and isoleucine in position 3 and 4 in the first repeat appear again in the same position in the third repeat and glutamines were found in position 1 and 2 of the second and the third cryptic repeat.

A dodecapeptide was synthesized comprising the amino acid sequence

Glu-Lys-Glu-Glu-Lys-Glu-Lys-Glu-Glu-Lys-Glu-Lys and the binding of antibodies in malarial sera from PNG to this peptide was tested by a radioimmunoassay (RIA). The malarial sera gave no signal in the RIA. This result was surprising because synthetic peptides corresponding to six other repeating sequences that have been determined in other antigens of P.falciparum all gave positive results (7, 8, 15).

In order to exclude sequencing errors the complete nucleotide sequence of Ag189 was again determined and an identical sequence and reading frame was obtained. In addition, the inserts of 3 further clones were sequenced, namely Ag126, Ag144 and Ag196 coding for segments of the same P.falciparum. These 3 clones are all in phase with β -gal, produce large fused polypeptides and exhibit the same open reading frame as Ag189. Therefore there is certainty about the reading frame. The sequence of Ag126, 144 and 196 includes in all 3 clones the region with the block of di- and tripeptide repeats. However differences among the 4 cDNA clones were also noted.

Ag144 which is 581 bp long commences at position 387 in relation to Ag189 and has deleted 6 bp in position 950 to 955, but contains 7 additional As at the 3' end of the cDNA, which codes for two more lysines. Ag126 and Ag196 are 451 and 452 bp long respectively and both start at position 458 in relation to Ag189. The Ag126- and Ag196-insert exhibit a deletion extending from position 901 to 955 in the sequence shown in Figure 8. It is believed that these deletions are artefacts of cloning in M13. Similar problems of maintaining cDNA

inserts in M13 have been observed with other malarial antigens (15).

Agl26 and Agl96 both differ from the sequence of Agl89 and Agl44 in two nucleotides. Agl26 and Agl96 contain at position 461 (in relation to Agl89) a "T" instead of an "A", replacing tyrosine by phenylalanine and in position 806 a "T" instead of "C", which has no effect at the amino acid level. Agl26 and Agl96 have 3 and 4 additional As at the 3' end coding for 1 and 2 more lysines, respectively.

Genomic organisation of ABRA

The insert of Agl26 was used in Southern blot experiments to investigate the genomic organisation of ABRA. DNA from 3 geographical isolates of P.falciparum, the homologous strain FC27 from Papua New Guinea, NF7 from Ghana and K1 from Thailand were restricted with EcoRI and AhaIII, size-fractionated on 1% agarose gels, blotted on nitrocellulose and probed with the ³²p-labelled insert of Agl26. As can be seen in Figure 9, the insert hybridized to a single 6.4 kb EcoRI fragment and a 1 kb AhaIII fragment in each isolate investigated. In addition, the DNAs of a further 3 isolates from Papua New Guinea (IMR143, IMR144 and MAD71) were probed with the 581 bp insert of Agl44 and showed identical fragment sizes in these isolates (data not shown).

III Other Antigens cloned in E.coli

Several other antigens of P.falciparum, which are natural immunogens in man (and therefore potential vaccine candidates), have been identified with antibodies raised against or affinity purified on

P.falciparum antigens expressed from cDNA sequences cloned in E.coli using the λ Amp3 vector. The clones, and the apparent molecular weights and stage specificities (determined by immunofluorescent microscopy) of the corresponding parasite antigens, are listed in Table 1.

Figure 10 is the nucleotide sequence of clone Ag169;

Figure 11 is the nucleotide sequence of clone Ag303;

Figure 12 is the nucleotide sequence of clone Ag358;

Figure 13 is the nucleotide sequence of clone Ag361;

Figure 15 is the nucleotide sequence of clone Ag394; and

Figure 15 shows indirect immunofluorescence on acetone-methanol fixed bloodstages of P.falciparum reacted with antibodies directed against antigens produced by Ag501 in bacteria T. - trophozoite (minimal reaction). S-schizont. G. - gametocyte (no reaction seen).

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TABLE 1

Clone	<u>Corresponding P.falciparum Antigen</u>	
	<u>Apparent Molecular Weight (Mr)*</u>	<u>Predominant Location by Immunofluorescence</u>
Ag169	N.A.	N.A.
Ag303 (Ag331) **	125,000-130,000	Schizonts
Ag358	Dominant bands are 210,000; 190,000 and 140,000	All stages
Ag361	70,000	Mature stages
Ag372	195,000; 140,000 and 80,000	Mature stages
Ag394	140,000***	All stages including rhoptry locations.
Ag501	~ 130,000	Mature stages

N.A. - Not available

* The apparent molecular weights (Mr) have been determined by Western blotting from 7.5% gels using antigens from the FC27 P.falciparum isolate. In some cases, the Mr can vary considerably in other isolates and of other gel conditions are employed. Also in some cases numerous other weaker bands are seen, presumably reflecting breakdown products or cross-reactions.

** It has been found that Ag303 and Ag331 correspond to fragments of the one coding sequence.

*** Cross-reactions with Ag23 and with bands of 105,000 and 102,000 were also observed.

MATERIALS AND METHODS

Parasites

P.falciparum isolates FCQ27/PNG (FC27), IMR143, IMR144 and MAD71 were obtained through the Papua New Guinea Institute of Medical Research. NF7 from Ghana, and K1 from Thailand, were obtained from D.Walliker, Edinburgh University. V1 from Vietnam was obtained from L.Miller, National Institute of Health, Bethesda, U.S.A. Parasites were maintained in asynchronous in vitro culture in Group O human erythrocytes according to Trager and Jensen (9). To obtain stage-specific life-cycle forms, parasite cultures were synchronised twice to within a six hour spread of maturation using sorbitol (10) and harvested at various time points of the asexual cycle. Naturally released merozoites were obtained as described previously (11).

Sera

Sera were obtained with informed consent from individuals living in the Madang region of Papua New Guinea. Some patients presented with acute malaria while in others, asymptomatic parasitaemia was detected in the course of routine surveys. Parasitaemic individuals were treated with chloroquine and convalescent serum was collected one or two weeks later. Parental consent was obtained before taking samples from children. In all cases, serum was separated and stored at -20°C for up to 12 months then held at -70°C. Presence or absence of splenomegaly was documented for some subsets and parasitaemia was assessed from a thick blood smear in all cases.

Clones expressing P.falciparum antigens

Methods for construction of the P.falciparum cDNA expression library and isolation of clones by antibody screening have been published (1). Replicas of the antigen-positive clones were grown overnight at 30°, induced at 38°, and lysed in situ as described (12). Individual human sera were pretreated to remove anti-E.coli activity, reacted with the colonies at a final dilution of 1:500 in 3% bovine serum albumin/Tris saline, pH 9.6 albumin, and the colonies then reacted with ¹²⁵I protein A from Staphylococcus aureus and autoradiographed as described (12).

Hybridization experiments

DNA carrying inserts were purified by CsCl centrifugation, digested with EcoRI, end-labelled with ³²P-dATP by the Klenow fragment of DNA polymerase I and size-fractionated on a 1% low-melting agarose-gel. The labelled inserts were recovered and hybridized to the bank of antigen-positive clones. In some cases the insert was first subcloned in the plasmid pUC-9 (13) purified by gel electrophoresis and then nick translated. Inserts which had been subcloned in this way were used in Southern blot experiments. For Southern blots, two micrograms of parasite DNA was digested with restriction, endonuclease according to the manufacturer's instructions, electrophoresed in a 1% agarose gel and blotted to nitrocellulose filters which were then hybridised with 10⁶ cpm/ml of the various probes.

Nucleotide sequence determination

The dideoxy chain termination method (14), was employed for sequence determination. The inserts of the various antigen-expressing clones and fragments generated by digestion with appropriate restriction endonucleases were cloned onto M13mp8 and/or M13mp9 (13).

Affinity purification of human antibodies against cloned malaria antigens

Induced 50ml cultures of antigen positive clones were prepared as described previously (15). The pelleted bacteria were sonicated and soluble proteins were conjugated to CNBr-activated Sepharose (Pharmacia, Sweden). Antibodies from a pool of human plasma were affinity-purified on the immobilised antigen as described (15).

Indirect immunofluorescence

Thin blood films of parasitized erythrocytes from asynchronous cultures of P.falciparum were fixed in 90% acetone/10% methanol and reacted with affinity-purified human antibodies. Sera from mice immunized with bacterial lysates of antigen-positive clones were also examined (16). Fluorescein-conjugated sheep anti-human Ig or sheep anti-mouse Ig antisera were used as the second antibody. Parasite nuclei were counterstained with propidium iodide and the slides were mounted in 90% glycerol/10% PBS containing p-phenylenediamine for viewing under U.V. illumination.

Immunoelectron Microscopy

Parasitized erythrocytes were fixed with glutaraldehyde, sectioned after being embedded in L.R.White resin and incubated with appropriately diluted
5 antibodies and protein A-gold using published procedures (11).

Immunoblotting

Merozoites and infected erythrocytes containing
10 either stage-specific or asynchronous parasites were diluted in sample buffer containing 3% SDS, 62.5 mM Tris-HCl, β -mercaptoethanol, pH 6.8 and heated for 2 min at 100°C. After centrifugation at 12,000g for 10 min., protein extracts were fractionated on 7.5% or 10%
15 polyacrylamide/SDS gels and transferred electrophoretically to nitrocellulose. Filters were blocked with 5% non-fat milk powder in phosphate-buffered saline (PBS) pH 7.4 and reacted with affinity purified human antibodies. They were then
20 incubated in 125 I-labelled protein A and autoradiographed.

In a separate experiment, parasitized cells and merozoites were first incubated in PBS containing 0.5%
25 Triton X-100, 5 mM PMSF, 1 mM TPCK, 2.5 mM EDTA and 2 mM iodo-acetamine for 30 mins at room temperature and centrifuged at 12,000g for 10 min. Supernatants and pellets were then individually diluted to equivalent final volumes in sample buffer and treated as before.

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15. Stahl, H-D., Crewther, P.E., Anders, R.F., Brown, G.V., Coppel, R.L., Bianco, A.E., Mitchell, F.G. and Kemp, D.J. (1985) Proc.Nat.Acad.Sci.USA 82, 543-547.
16. Coppel, R.L., Brown, G.V., Mitchell, G.F., Anders, R.F. and Kemp, D.J. (1984) EMBO J. 3, 403-407.

CLAIMS:

1. A DNA molecule comprising a nucleotide sequence substantially corresponding to all or a portion of the base sequence coding for an antigen of P.falciparum selected from the group consisting of the Acidic Basic Repeat Antigen Rhoptry (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith.
2. A DNA molecule according to claim 1, wherein said nucleotide sequence is characterised by at least a portion thereof comprising a base sequence substantially as shown in Figure 4, Figure 8, or Figures 10 to 14.
3. A DNA molecule comprising a nucleotide sequence capable of being expressed as at least one polypeptide displaying the antigenicity of an antigen of P.falciparum selected from the group consisting of the Acidic Basic Repeat Antigen Rhoptry (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith.
4. A recombinant DNA molecule comprising a nucleotide sequence according of any one of claims 1 to 3, operatively linked to an expression control sequence.
5. A recombinant DNA cloning vehicle or vector capable of expressing all or a portion of at least one polypeptide or protein of P.falciparum, and having inserted therein a nucleotide sequence according to any one of claims 1 to 3, said sequence being operatively linked to an expression control sequence.

6. A recombinant DNA cloning vehicle or vector according to claim 5, characterised in that said nucleotide sequence and said expression control sequence are inserted into a bacteriophage.
7. A host cell containing a recombinant DNA molecule according to claim 4, or a recombinant DNA cloning vehicle or vector according to claim 5.
8. A synthetic peptide or polypeptide displaying the antigenicity of all or a portion of an antigen of P.falciparum selected from the group consisting of the Acidic Basic Repeat Antigen Rhoptry (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith.
9. A fused polypeptide comprising a polypeptide sequence displaying the antigenicity of an antigen of P.falciparum selected from the group consisting of the Acidic Basic Repeat Antigen Rhoptry (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith as the C-terminal sequence, and an additional polypeptide as the N-terminal sequence fused thereto.
10. A fused polypeptide according to claim 9, wherein the additional polypeptide is a polypeptide coded for by the DNA of a recombinant DNA cloning vehicle or vector.
11. A composition for stimulating immune responses against P.falciparum antigens in a mammal, comprising at

least one polypeptide displaying the antigenicity of an antigen of P.falciparum selected from the group consisting of the Acidic Basic Repeat Antigen Rhoptry (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith, together with a pharmaceutically acceptable carrier therefor.

12. A method of stimulating immune responses against P.falciparum antigens in a mammal, which comprises administering a composition according to claim 11 to said mammal.

AMENDED CLAIMS

[received by the International Bureau on 26 May 1987 (26.05.87);
original claims 1-12 replaced by amended claims 1-12 (3 pages)]

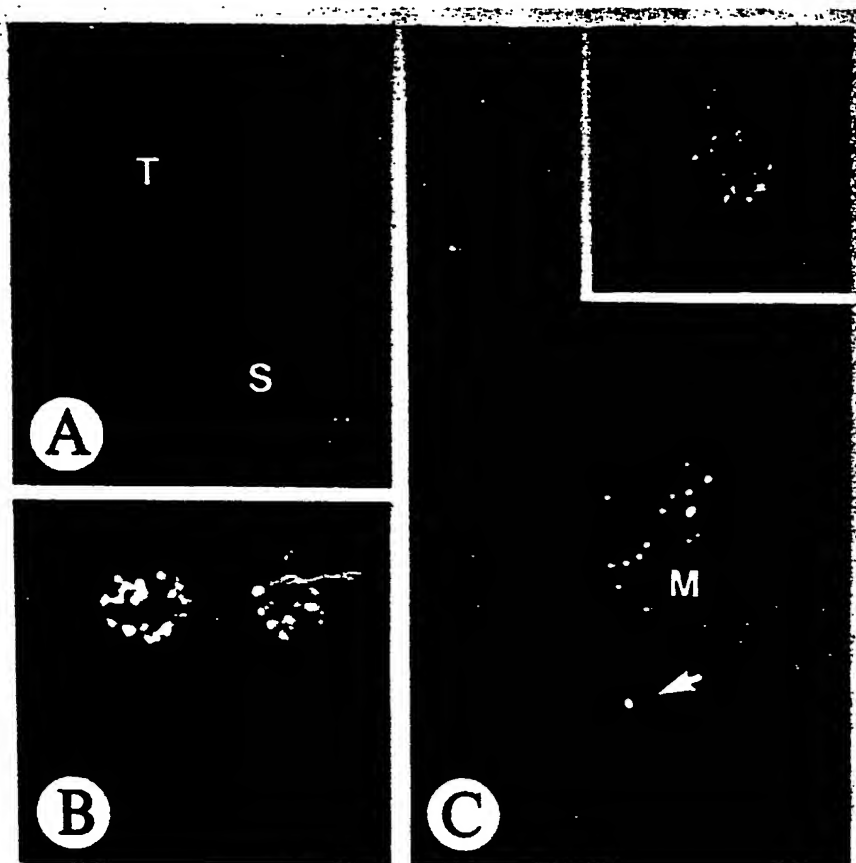
1. A DNA molecule comprising a nucleotide sequence substantially corresponding to all or a portion of the base sequence coding for an antigen of P.falciparum selected from the group consisting of the Rhoptry Protein, the Acidic Basic Repeat Antigen (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith.
2. A DNA molecule according to claim 1, wherein said nucleotide sequence is characterised by at least a portion thereof comprising a base sequence substantially as shown in Figure 4, Figure 8, or Figures 10 to 14.
3. A DNA molecule comprising a nucleotide sequence capable of being expressed as at least one polypeptide displaying the antigenicity of an antigen of P.falciparum selected from the group consisting of the Rhoptry Protein, the Acidic Basic Repeat Antigen (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith.
4. A recombinant DNA molecule comprising a nucleotide sequence according of any one of claims 1 to 3, operatively linked to an expression control sequence.
5. A recombinant DNA cloning vehicle or vector capable of expressing all or a portion of at least one polypeptide or protein of P.falciparum, and having inserted therein a nucleotide sequence according to any one of claims 1 to 3, said sequence being operatively linked to an expression control sequence.

6. A recombinant DNA cloning vehicle or vector according to claim 5, characterised in that said nucleotide sequence and said expression control sequence are inserted into a bacteriophage.
7. A host cell containing a recombinant DNA molecule according to claim 4, or a recombinant DNA cloning vehicle or vector according to claim 5.
8. A synthetic peptide or polypeptide displaying the antigenicity of all or a portion of an antigen of P.falciparum selected from the group consisting of the Rhoptry Protein, the Acidic Basic Repeat Antigen (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith.
9. A fused polypeptide comprising a polypeptide sequence displaying the antigenicity of an antigen of P.falciparum selected from the group consisting of the Rhoptry Protein, the Acidic Basic Repeat Antigen (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith as the C-terminal sequence, and an additional polypeptide as the N-terminal sequence fused thereto.
10. A fused polypeptide according to claim 9, wherein the additional polypeptide is a polypeptide coded for by the DNA of a recombinant DNA cloning vehicle or vector.
11. A composition for stimulating immune responses against P.falciparum antigens in a mammal, comprising at

least one polypeptide displaying the antigenicity of an antigen of P.falciparum selected from the group consisting of the Rhoptry Protein, the Acidic Basic Repeat Antigen (ABRA), the antigen of any of clones Ag169, Ag303, Ag358, Ag361, Ag372, Ag394 or Ag501, defined herein, and other antigens of P.falciparum cross-reactive therewith, together with a pharmaceutically acceptable carrier therefor.

12. A method of stimulating immune responses against P.falciparum antigens in a mammal, which comprises administering a composition according to claim 11 to said mammal.

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*Fig.1.**FIG.2.*

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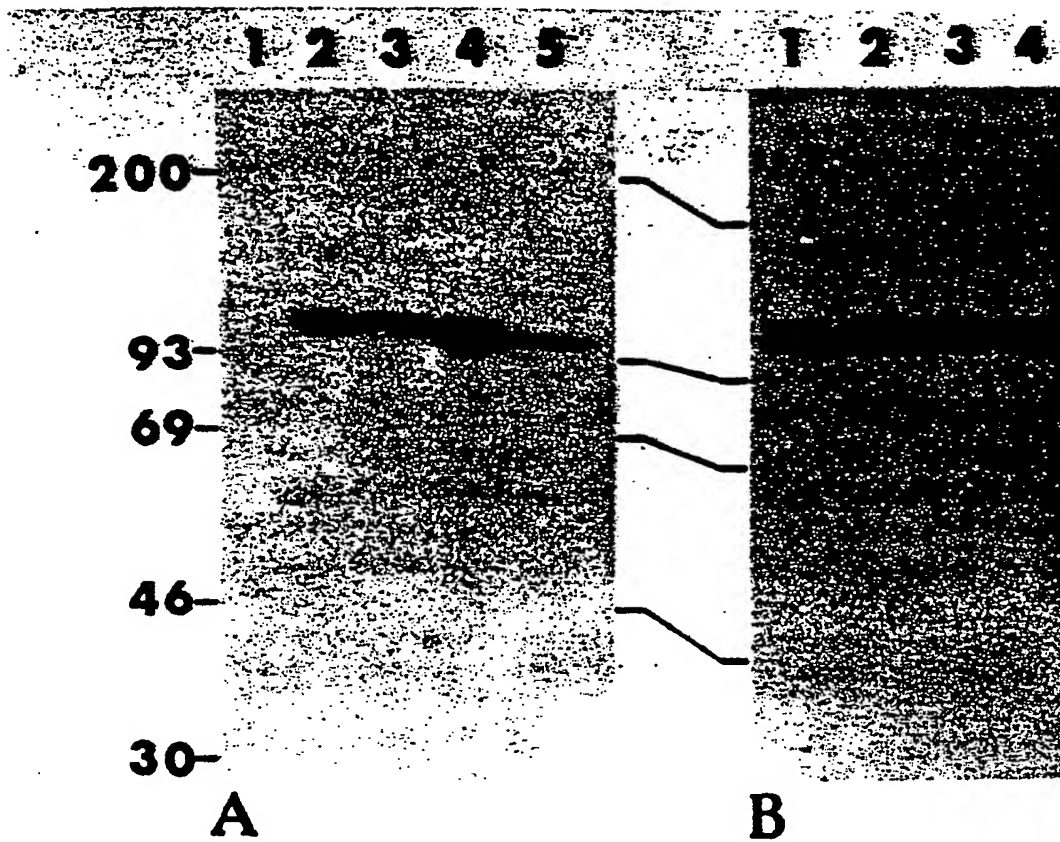


FIG.3.

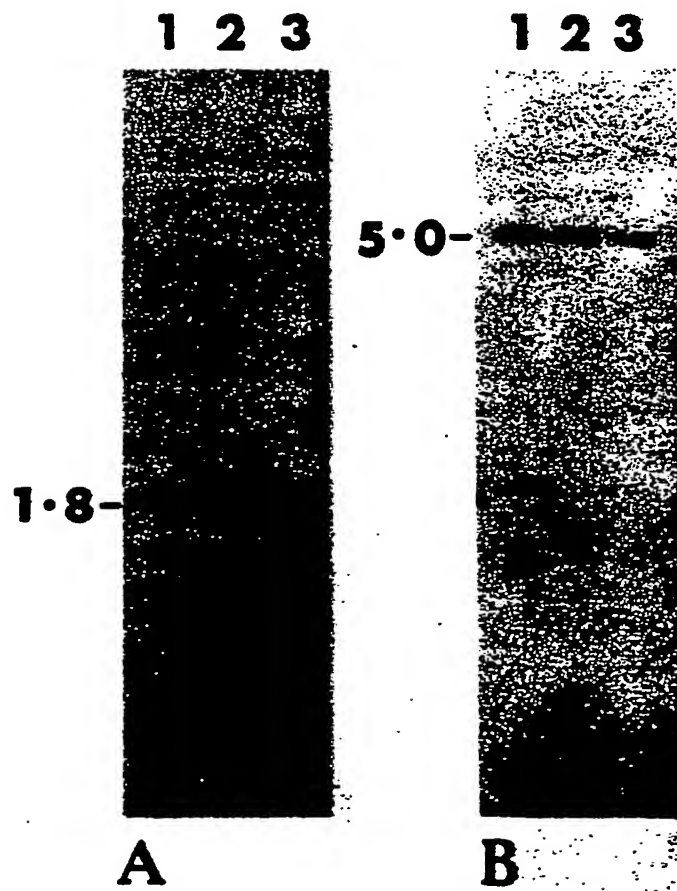


FIG.5.

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ValAsp IleLeuGluGluLysThrLysAspGlnAspLeuGluIleGluLeuTyrLysTyr
 GCGTTGACATATTAGAAAGAAAACCAAGGATCAAGATTTAGAAATAGAAATTATACAAATAT
 10 20 30 40 50 60
 MetGlyProLeuLysGluGlnSerLysSerThrSerAlaAlaSerThrSerAspGluLeuSer
 ATGGGACCATTAAGAGAACATCTAAAGTACAAGTGTGCATCTACTAGTGATGAATTATC
 72 82 92 102 112 122
 GlySerGluGlyProSerThrGluSerThrSerThrGlyAsnGlnGlyGluAspLysThrThr
 AGGTTCTGAAGGTCCATCTACTGAATCTACAAGTACAGGAAATCAAGGTGGAAGATAAAACAA
 134 144 154 164 174 184
 AspAsnThrTyrLysGluMetGluGluLeuGluGluAlaGluGlyThrSerAsnLeuLys
 CAGATAATACATACAAAGAAATGGAAAGATTAGAAAGAGCTGAAGGAACCTTCAAACTTTAAA
 196 206 216 226 236 246
 LysGlyLeuGluPheTyrLysSerSerLeuLysLeuAspGlnLeuAspLysGluLysProLys
 AAAGGTTTAGAAATTTTATAAATCTTCTCTAAAACTTGATCAATTAGATAAAGAAAAACCTAA
 258 268 278 288 298 308
 LysLysLysSerLysArgLysLysLysArgAspSerSerSerAspArgIleLeuLeuGluGlu
 AAAGAAAAATCTAAAGAAAAAAAGAGAGACAGTTCTAGTGACAGAAATATTATTAGAAG
 320 330 340 350 360 370
 SerLysThrPheThrSerGluAsnGluLeu***
 AATCTAAACCTTTACTTCTCAAAATGAATTGTAAATTAATAATTAATCTACATGTAGAT
 382 392 402 412 422 432
 TTTATTATATACATCATGTAAATCATATTATAGAAATTTATTTTAAAGAAAAAAAAAA
 444 454 464 474 484 494

FIG. 4.

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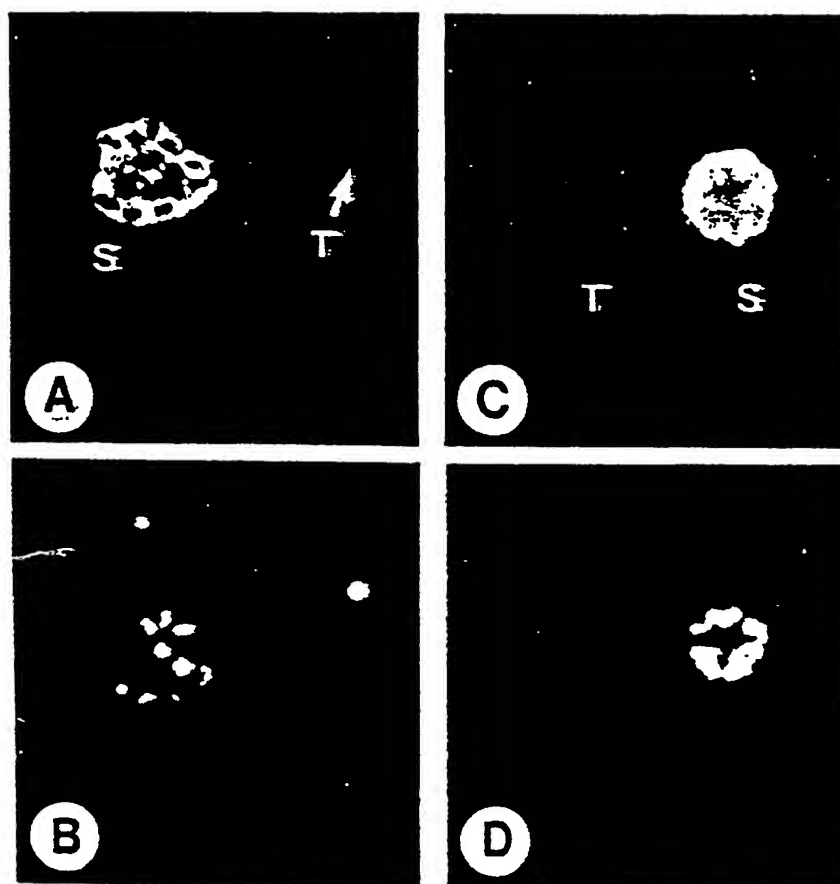


Fig.6.

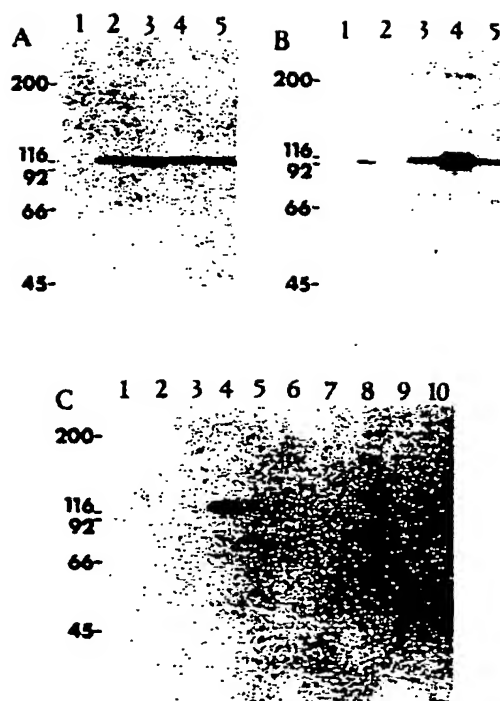


Fig.7.

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HisTyrLysLysArgLysAlaGlnGluLysGlyLeuProGluPro
 CATTATAAGAAAGAAAGCTCAAGAAAAAGGATTAC CAGAACCTACTGTTACTAATGAA 60
 10 20 30 40 50

GluTyrValGluGluLeuLysLysGlyIleLeuAspMetGlyIleLysLeuLeuPheSer
 GAATA TGTGGAAGAAATTAAAGAAAGGATTCTAGATATGGGTATCAAAATTATTATTAGT 120
 70 80 90 100 110

LysValLysSerLeuLeuLysLysLeuLysAsnLysIlePheProLysLysLysGluAsp
 AAAGTTAAAGCC TATTAAAAAATTAAAAAATAAAA TATTCCTAAGAAAAAAGAGAT 180
 130 140 150 160 170

AsnGlnAlaValAspThrLysSerMetGluGluProLysValLysAlaGlnProAlaLeu
 AATCAAGCAGTAGATACCAAAGTATGGAAAGAACCCCAAAGTTAAAGCACCAACGCTCTT 240
 190 200 210 220 230

ArgGlyValGluProThrGluAspSerAsnIleMetAsnSerIleAsnAsnValMetAsp
 AGAGGTGTTGAACCAACGGGAAGATTCTAATA TTATGAACAGTATTAAATAATGTTATGGAT 300
 250 260 270 280 290

GluIleAspPhePheGluLysGluLeuIleGluAsnAsnAsnThrProAsnValValPro
 GAAATTGATTCTTTTGAAAAAGAA TTAATCGAAAA TAAATAATACACCTAA TGTGTGTACCA 360
 310 320 330 340 350

 FIG.8A.

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ProThrGlnSerLysLysLysAsnLysAsnGluThrValSerGlyMetAspGluAsnPhe
 CCAACTCAATCAAAAAAAAAAACAATAATGAACTGTATCTGGTATGGATGAAAAATTTT
 370 380 390 400 410 420
 Ag 144

AspAsnHisProGluAsnTyrPheLysGluGluTyrTyrTyrAspGluAsnAspAspMet
 GATAATCATCCTGAAAAATTATTTTAAAGAGAAATAATTTATGATGAAAAATGATGATATG
 430 440 450 460 470 480
 Ag 126 / Ag 196

GluValLysValLysLysIleGlyValThrLeuLysLysPheGluProLeuLysAsnGly
 GAAGTAAAGTTAAAAAATAGGTGTCACATTAAAAAATTTGAACCACTTAAAAAATGGA
 490 500 510 520 530 540

AsnValSerGluTyrIleLysLeuIleHisLeuGlyAsnLysAspLysLysHisIleGlu
 AATGTTAGTGAACCATTAATAATTGATTTCATTAGGAAATAAAGATAAAAAACACATTGAA
 550 560 570 580 590 600

AlaIleAsnAsnAspIleGlnIleIleLysGlnGluLeuGlnAlaIleTyrAsnGluLeu
 GCTATAAACACACGATATTCAAATTATTAAACAAGAAATTACAAGCTATTTATAATGAACCTT
 610 620 630 640 650 660

MetAsnTyrThrAsnGlyAsnLysAsnIleGlnGlnIlePheGlnGlnAsnIleLeuGlu
 ATGAATTATACAAATGGAAACAAAAATATTCACAAATATTTCAACAAAAATATCTAGAA
 670 680 690 700 710 720

Fig. 8B.

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AsnAspValLeuAsnGlnGluThrGluGluGluMetGluLysGlnValGluAlaIleThr
AATGATGTTCTTAATCAAGAAACGGAGGAAGAAATGGAAAAACAAGTTGAAGCAATCACCC
730740750760770780

LysGlnIleGluAlaGluValAspAlaLeuAlaProLysAsnLysGluGluGluGluLys
AAGCAAAATAGAGCTGAAGTGGATGCCCTCGCACCAAAAAATAAGGAAGAGAAAGAAAAA
790800810820830840

GluLysGluLysGluLysGluLysGluLysGluLysGluLysGluLysGluLysGluLys
GAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAA
850860870880890900

GluGluLysGluLysGluGluLysGluLysGluGluLysGluGluLysGluGluGluLys
GAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAAGAAAAA
910920930940950960

LysAsn
AAAAA

Fig.8C.

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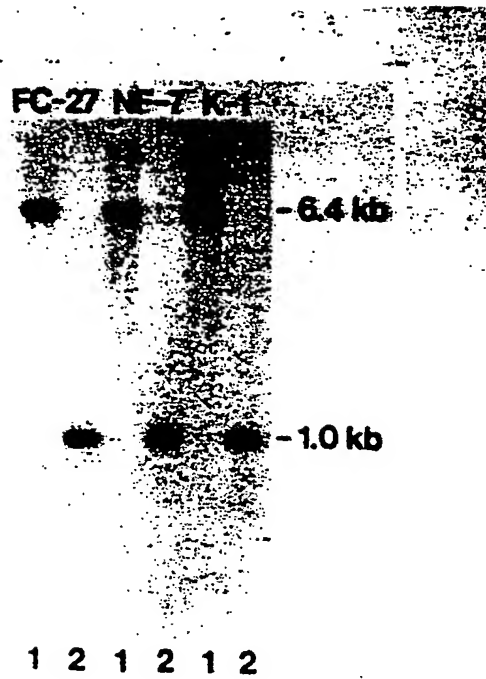


Fig. 9.

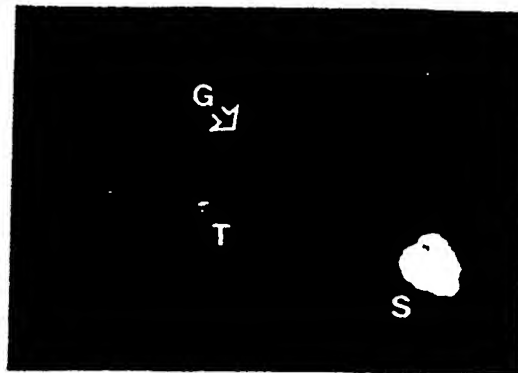


Fig. 15.

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[illegible]

	80	90	100
TCACTTCTTGTAACCTCTTAGATTCTTCT			
	280	290	300
TTCTTCAACTTTTGATTTAACCATAATGT			
	480	490	500
CTTTTTTGGCTTTTAAAGTTTCCTTAT			
	680	690	700
GTTACGTC TGTCAA TGGA CTGGTA ATGGT			
	880	890	900

FIG. 10A.

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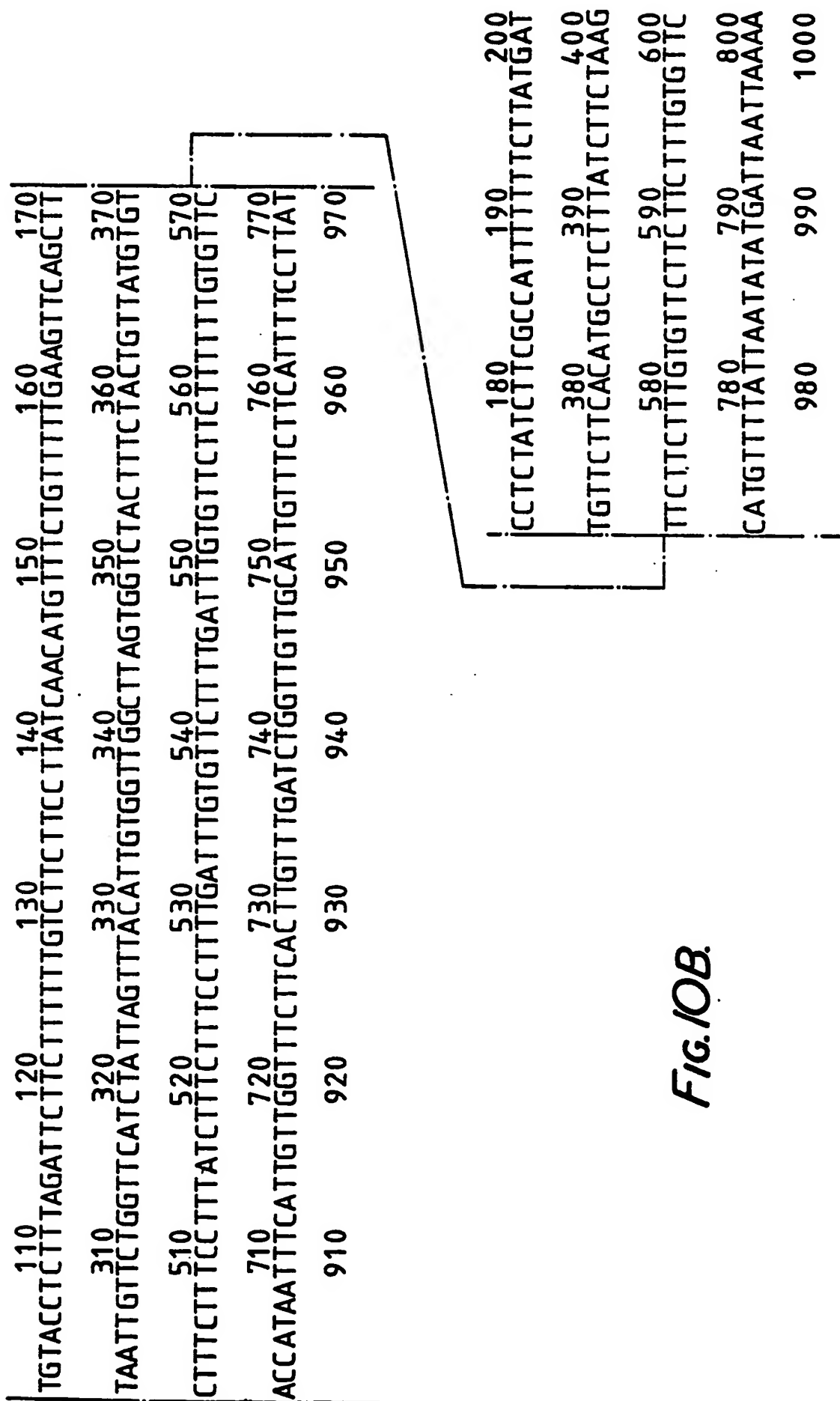


FIG. 10B.

¹⁰GAATTCCGAG ²⁰AGCTCAAAGT ³⁰TCTAGTTTCC ⁴⁰AGTTCTAGTT ⁵⁰CAAGTTCAGA ⁶⁰AAGTCTTCCCT ⁷⁰GCTAATGGAC CT
⁸²GATTCCCCCTA ⁹²CTGTTAAACC ¹⁰²GCCAAGAAAT ¹¹²TTACAAAATA ¹²²TATGTGAAAC ¹³²TGGAAAAAAC ¹⁴²TTCAAGTTCC TA
¹⁵⁴GTATATATTA ¹⁶⁴AGGAGAATAC ¹⁷⁴ATTAATACCT ¹⁸⁴AAATGGAAAG ¹⁹⁴TATACGGAGA ²⁰⁴AACAAAAGAA ²¹⁴TACTACTGAA AA
²²⁶TAACAAAGTT G ²³⁶ ²⁴⁶ ²⁵⁶ ²⁶⁶ ²⁷⁶ ²⁸⁶

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Fig.11.

AATAAT-CAAAACAATAATGGAAAGTGTAACATATATATAATAATCAAGCGATTTAATAA
ATAACTATTTTAATAATAATAATAATAATAATAAACAATAATGCCAAATATATTCTAGT
GAATATGTACA-GAACATATTATAACACACATATGAATCATTTCATAATGATAATAAGGAA
TTATCATATTGATGATTCAAAAGAAGTGTAAATTAT

Fig.12.

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GAATTCTTTAATGGTAAAGAACCAATAGAGGTATAAATCCTTATGAAGCTGTTGCTTATGGTGCTGCTA 70
140 CATTAACTTTAGGTATAGAAACTGTGGTGGTATTATGACACAAATTAATTAAGAAGAAATACGTGCATCCC 200
270 TTTTGAAGGAGAAAGAGCATTAACCAAGATAATCACCTTTAGGAAAGTTTGAATTATCTGGTATTCCA 330
400 TTACATGTTGAAGCTGAAGACAAAGGTACAGGTAAAAGTAGAGGTATACTATTACTAATGACAAAGGTAG 460
530 AACTTAAGAGAAAGTTGAAGGCCAAAATAACCTGATAATTATATACAGAGTATGAAAGCAACTGTTGAA 590
660 TGTTAAAGATGTTGAAGATTGGTTAAATAATAACTCGAAATGTTGATTCGAAAGCATTAAAAACAAAAATTA 720
790 CTTCAACCAACCTAGTGGAGACGAAGATGTAGATAGTGACGAATTATAAAATCTTCACATTTTATGAAT 850
920 TTTAAACAAATTAAAAAAATAACATATATATGTATATATATATATATATATATATATATATATATATAT 980

Fig 13A.

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80 90 100 110 120 130
 TCCAAGCAGGTATTATTTTAGGTGAAGAAATTACAAGACGTTGTTTATTATAGATGTTACTC
 210 220 230 240 250 260
 AACCAAAAAATCACAAACCTTTTCAACATATCAAGATAACCACCACGTCTTAATTCACT
 340 350 360 370 380 390
 CCACCACAAAGAGGAGTACCCAAAATTGAAGTTACCTTTACCGTAGACAAAAATGGTATC
 470 480 490 500 510 520
 ATTATCGAAAGAACCAATCGAAAAAATGATGAATGCAGAAAAATTCGCAGTTGAGATAA
 600 610 620 630 640 650
 GATAAAGATAAATTAGCTGATAAAATCGAAAAAGAAGATAAAAAATACTATCCTTTTCAGC
 730 740 750 760 770 780
 AAAGATCTTGAAGCTGTATGCCAACCAATCATGTTAAATTATATGGTCAACCAAGGAGGAC
 860 870 880 890 900 910
 ATATATATTTATATATGTAATATATTTATGCATATATTA TGAATTACCTTCTTTT
 990 1000 1010 1020 1030 1040
 AGTTAAATGTATATATAAAAAAAACGGAATTC

FIG. 13B.

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10	20	30	40	50	60
AATCCGTAC	TAATGTTGTA	ACACCACTTA	TCATACAAGC	ACAATCAGTA	ATGCACICTA
130	140	150	160	170	180
TAGATTGGGA	TTTGTTGAA	GGATTGTTGT	GTCGTAAAAA	TGAATTGCCA	TATTIGAAAA
250	260	270	280	290	300
AATCCAATGG	TTCAGAGAAA	AGTAGTAATA	AACAAAAATA	TAATGAATCT	GATAAAAGAG
370	380	390	400	410	420
TAAAAGAAAA	AGCTAAAACA	CTTGGGTAA	GTATTATCGT	ATTTGATAAT	ATGACAGAGA
490	500	510	520	530	540
ATACATCTGG	AACATCTGGG	AAACCCAAAG	GTGTTATGTT	AAGCAATAGG	AATTGTGATA
610	620	630	640	650	660
TAICTTATTT	ACCCGTATCT	CATATATATG	AAAGGGTAT	TTTTTTTCATT	GCTTTGTTTT
730	740	750	760	770	780
ATTCAAAAGC	TGAAATTATA	TTAGGAGTAC	CCAAAGTTTT	TAATAGAAIG	TATGCAACTA
850	860	870	880	890	900
ATTIACGTAA	AGGTAAAAAT	AATGGAAATT	TCAGTAAAGT	TGTTGAAGGT	ATTACTAATA
970	980	990	1000	1010	1020
GGAAATTATC	TCCAGAGGTT	GCTGAGGGTT	TAAGTGTCT	ATTAAATGTT	AAGTATTATC

Fig.14A.

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70	AATTAGTAT	80	AGATAATAATT	90	ATTGATAIAT	100	TAAATAATAC	110	AAAATTAGAA	120	TGGTTGTGTT
190	AGCTGATAAT	200	TTTAGATAAT	210	CTAACTAAGC	220	GTAGTGAAAT	230	GAAGATAGAA	240	AATGAAGAAA
310	AAGACATTAG	320	TTTGTTGCCC	330	TTAGAATGTG	340	ATAAGGAAAA	350	AATAGAAAAG	360	ATTAATTTCAT
430	ATAAAATAGC	440	CAATGTTACT	450	GTTCAAAACG	460	AAGATCCCTAA	470	TTTTATTGCC	480	TCTATTGTGT
550	ATGGTGTAAT	560	ACCTCCCATGT	570	GATTGTAATA	580	TAATAAAGAA	590	ATATCCTCTA	600	ACAACACATT
670	TGGGTGTAAA	680	GATAAATATA	690	TGGAGTAGAG	700	ATATAAAAT	710	TTTGAATACA	720	GACATAATGTA
790	TTATGACGAA	800	AATAAATAAT	810	TTATCACGTT	820	GTAAGGAAGTG	830	GATAGCAAAA	840	CAGGCTATAA
910	TATCAAGAAA	920	AATAAAAGAT	930	AAGATAAACC	940	CTAATATGGA	950	TGTTATCCTA	960	AATGGAGGTG
1030	AAGGATAIGG	1040	TTTAACGGAA	1050	TCTACGGGTC	1060	CCATATTTT	1070	ACAAGAIGTA	1080	GATGACTGTA

FIG. 4B.

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1090	1100	1110	1120	1130	1140
ACACTGAAAG	TATGGGAGTA	GCIGTTTCTC	CTAGTACAAG	ATACAAAGTA	AGAACATGGG
1210	1220	1230	1240	1250	1260
TGTTTAGTGG	ATACTTTTAA	GAAAAGGAAT	CTACAGAACA	TGCTTTCAGG	AATGATGGTT
1330	1340	1350	1360	1370	1380
GATCAAAAGG	TTTGGTTAAA	TTATCTCAAG	GTGAATAAT	AGAAACTGAA	ATGATAAATA
1450	1460	1470	1480	1490	1500
ATGGACCATT	GGGAATTATA	TCTGTGGACA	AACATAAAT	ATTACATTT	TTAAAAAATG
1570	1580	1590	1600	1610	1620
AAACATTAAA	TGATCCTATT	TATGTTGATT	ATGTAAAGGG	AAAAATGATG	GAAATTTATA
1690	1700	1710	1720	1730	1740
GGGACACTAC	AAACTACCTT	ACTCCAACAT	TAAAAATAAG	AAGATTCAAT	GTATTTAAAG
1810	1820	1830	1840	1850	1860
GCACGGGTAG	TATGAATAAT	GGTAAAAGTG	GAAGTAAATC	TGATATTAAA	GGTGGAAGTA
1930	1940	1950	1960	1970	1980
AAAGTGGAAG	TAAAGATGAT	ATAAAAAGTG	GAAGTAAAGA	TCATATAAAA	CGGAATT

SUBSTITUTE SHEET

FIG. 14C.

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1150	1160	1170	1180	1190	1200
AAATTTATAA	GGCTACAGAT	ACTATACCAA	AAGGAGAATT	GTAAATTAAA	AGTGATTCTA
1270	1280	1290	1300	1310	1320
ATTTTAAAC	GGGAGATATT	GTACAAATTA	ATGATAATGG	TTCCTTAACA	TTTTTAGATA
1390	1400	1410	1420	1430	1440
ATTTATATTC	CCAAATCCCT	TTTGTAATTT	TTTGTTGGC	ATATGGTGAT	GATTCATGG
1510	1520	1530	1540	1550	1560
ATAATATGTT	AAAGACAAC	GGTGATAGATG	AGAAAAATTT	TTCAGAAAAA	TTAATTGATG
1630	1640	1650	1660	1670	1680
AAAAAACTAA	TTTAAATAGA	TACAATGTTA	TTAATGACAT	ATACTTAACT	TCCAAACCAT
1750	1760	1770	1780	1790	1800
ATTTTCTTT	TTTTATAGAT	GAAGTTAAAA	AGAAATATGA	AGAAAAATTA	AGTGAAGTAA
1870	1880	1890	1900	1910	1920
AAGATGATAT	AAAAAGTGGG	AGTAAAGATG	ATATAAAAAG	TGGAAGTAAA	GCTGATATAA
1990	2000	2010	2020	2030	2040

FIG. 14D.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 86/00386

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC Int. Cl. ⁴ C07H 21/04, C12N 1/20, C07G 17/00, C07K 13/00, 15/12, A61K 39/015 // C12R 1/19, C12N 15/00, C12P 21/02						
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px; vertical-align: top;"> IPC US </td> <td style="padding: 5px; vertical-align: top;"> WPI and WPIL) KEYWORD USPA, USP77, USP70) Plasmodium falciparum </td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *</div> <p style="padding: 10px 0 0 40px;">C.A KEYWORD: Plasmodium falciparum</p>			Classification System	Classification Symbols	IPC US	WPI and WPIL) KEYWORD USPA, USP77, USP70) Plasmodium falciparum
Classification System	Classification Symbols					
IPC US	WPI and WPIL) KEYWORD USPA, USP77, USP70) Plasmodium falciparum					
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹						
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³				
P	WO 86/06075 - (THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH) 16 October 1986 (16.10.86)	1-12				
P	Molecular and Biochemical Parasitology, Volume 18, issued 1986, L. Schofield et al. "A Rhoptry antigen of Plasmodium falciparum contains conserved and variable epitopes recognized by inhibitory monoclonal antibodies", see pages 183-195	1-12				
A	Proceedings of the National Academy of Science U.S.A., Volume 77, Number 6, issued June 1980 (06.80), A. Kilejian. "Stage-specific proteins and glycoproteins of Plasmodium falciparum: identification of antigens unique to schizonts and merozoites", see pages 3695-3699	1-12				
A	Molecular and Cellular Biology, Volume 6, Number 3, issued March 1986 (03.86), R.T. Schwarz et al. "Structural diversity of the major surface antigen of Plasmodium falciparum Merozoites"; see pages 964-968	1-12				
(continued)						
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>						
IV. CERTIFICATION						
Date of the Actual Completion of the International Search 18 March 1987 (18.03.87)	Date of Mailing of this International Search Report 2 April 1987 (2-4-87)					
International Searching Authority Australian Patent Office	Signature of Authorized Officer J.W. ASHMAN					

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Nature, Volume 317, issued 19 September 1985 (19.09.85), A.A. Holder et al. "Primary structure of the precursor to the three major surface antigens of Plasmodium falciparum merozoites", see pages 270-273	1-12
A	Biochemistry, Volume 81, issued June 1984 (06.84), M.J. McGarvey et al. "Identification and expression in Escherichia coli of merozoite stage-specific genes of the human malarial parasite Plasmodium falciparum", see pages 3690-3694	1-12
A	AU,A, 47326/85 (THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH) 20 March 1986 (20.03.86)	1-12
A	AU,A, 39046/85 (THE WELLCOME FOUNDATION LIMITED) 5 September 1985 (05.09.85)	1-12
A	GB,A, 2096893 (THE WELLCOME FOUNDATION LIMITED) 27 October 1982 (27.10.82)	1-12

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

Claim 1 has a plurality of inventions, in that antigen ABRA has no unifying elements in common with antigens AG 169, 303, 358, 361, 372, 394 or Ag 501.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Search restricted to antigen ABRA

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 86/00386

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Members			
WO 8606075	IL 78476 ZW 8386	AU 56037/86	ZA 8602732		
AU 47326/85	EP 193586 WO 8601802 ZA 8506960	GB 2176191 ZW 14985	IL 76338 GB 8610243		
AU 39046/85	ZW 2485 GB 8404692 HU 37460 ZA 8501334	DK 799/85 GB 8504429 IL 74409 GB 8424340	EP 154454 GB 2154592 JP 61019490		
GB 2096893	AU 82593/82 IL 65496 ZW 7282	CA 1196282 JP 58010524	EP 62924 ZA 8202539		

END OF ANNEX

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